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[RUSH] **MESSAGE:**

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A.PE page 1 of 1 (05-28-02)
SPEC page 1 of 1 (05-28-02)
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Thank you

[XRUSH] **RESPONSE:**

INITIALS:

NOTE: This form will be included as part of the official USPTO record, with the Response document coded as XRUSH.

REV 10/04

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PATENT

Atto · Reference Number 245-62107
Application Number 10/053,243

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Giovannoni *et al.*

Art Unit: 1656

Application No. 10/053,243

Filed: January 16, 2002

For: HIGH-THROUGHPUT MICROBIAL CULTURING

Examiner: Not yet assigned

Date: May 10, 2002

CERTIFICATE OF MAILING

I hereby certify that this paper and the documents referred to as being attached or enclosed herewith are being deposited with the United States Postal Service on May 10, 2002 as First Class Mail in an envelope addressed to: BOX MISSING PARTS, COMMISSIONER FOR PATENTS, WASHINGTON, D.C. 20231.

Tanya M. Harding, Ph.D.
Attorney for Applicant

BOX MISSING PARTS
COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SECOND PRELIMINARY AMENDMENT

Prior to calculating the filing fee, please enter the following amendments in the above-referenced application:

In the Specification:

Please insert the following header and paragraph on page one immediately before line 6:

ACKNOWLEDGMENT OF GOVERNMENT SUPPORT

This invention was made with government support under National Science Foundation Major Research Instrumentation Grant, No. OIA-9977469. The government has certain rights in this invention.

Please replace the paragraph at Page 9, lines 16-23, with the following:

In certain embodiments, cells can also be cultured on conventional growth media, to compare growth rates, culturability, or like parameters. For instance, a sample of the raw source material may be used as a starting culture for growth on nutrient agar plates. In other instances, a portion of some or all of the microbial cultures that show growth in the micro-culture compartments (*e.g.*, the compartments of a microtiter dish) can be used to inoculate traditional

medium (either liquid medium or nutrient agar plates, for instance), in order to examine whether the microbes will grow under traditional conditions.

Please replace the paragraph at Page 10, lines 12-19, with the following:

Within an array, each arrayed cell sample or mixture of cells is addressable, in that its location can be reliably and consistently determined within at least the two dimensions of the array surface. Thus, in ordered arrays the location of each cell sample is assigned to the sample at the time when it is spotted onto the array surface and usually a key is provided in order to correlate each location with the appropriate "target" cell sample. Often, ordered arrays are arranged in a symmetrical grid pattern, but samples could be arranged in other patterns (*e.g.*, in radially distributed lines or ordered clusters).

Please replace the paragraph at Page 11, line 29 through Page 12, line 3, with the following:

Sample spots on macroarrays are of a relatively large size, for instance large enough to permit detection of a hybridization signal without the assistance of a microscope or other sophisticated enlargement equipment. Thus, spots may be as small as about 0.1 mm across, with a separation of about the same distance, and can be larger. Larger sample spots on macroarrays, for example, may be about 0.5, 1, 2, 3, 5, 7, or 10 mm across. Even larger spots may be larger than 10 mm (1 cm) across, in certain specific embodiments. The array size will in general be correlated to the size of the sample spots applied to the array, in that larger spots will usually be found on larger arrays, while smaller spots may be found on smaller arrays. This correlation is not necessary to the invention, though.

Please replace the paragraph at Page 21, lines 6-11, with the following:

Certain examples of automated array readers (scanners) will be controlled by a computer and software programmed to direct the individual components of the reader (*e.g.*, mechanical components such as motors, analysis components such as signal interpretation and background subtraction). Optionally software may also be provided to control a graphic user interface and one or more systems for sorting, categorizing, storing, analyzing, or otherwise processing the data output of the reader.

Please replace the paragraph at Page 24, lines 25-28, with the following:

To prepare the frozen dilution cultures for lysis and concentration, the tubes were placed into a 95 °C heat block for five minutes, then put in –80 °C for 15 minutes. This process, which helps insure cell lysis, was repeated once, and the tubes finally thawed at room temperature.

In the Claims:

Please amend the claims to read as follows:

1. (Amended) A method of isolating and identifying a microbial species from a source environment, comprising:

gathering from the source environment a sample suspected of containing at least one microorganism that has not been cultured using standard culturing techniques;

providing a volume of culture medium to the microorganism in at least one microtiter plate compartment;

incubating the microorganism in the medium for a period of time and in an environment sufficient to result in growth of the microorganism if the medium and environment are capable of supporting such growth to produce a culture sample, wherein growth of the microorganism comprises an increase in the number of microorganisms in the compartment to no more than about 5×10^4 cells/milliliter;

detecting growth of the microorganism using an automated detection method that comprises removing a portion of the culture sample and depositing the portion onto a surface, wherein growth of the microorganism indicates that the microbial species has been isolated from the source environment; and

identifying the microbial species, wherein identifying the microorganism includes hybridization of a probe to a nucleic acid molecule of the microorganism amplification of a nucleic acid molecule of the microorganism; immunodetection of a molecule of the microorganism; sequencing of a nucleic acid molecule of the microorganism; or a combination of two or more thereof.

2. (Reiterated) The method of claim 1, wherein a plurality of individual microorganisms are separately incubated in microtiter plate compartments.

3. (Reiterated) The method of claim 2, wherein the plurality is at least 20.

4. (Reiterated) The method of claim 2, wherein the plurality is at least 50.

5. (Reiterated) The method of claim 2, wherein the plurality is at least 100.

6. (Reiterated) The method of claim 2, wherein the plurality is at least 400.

7. (Reiterated) The method of claim 2, wherein the plurality is at least 1000.

8. (Reiterated) The method of claim 2, wherein the plurality is at least 1500.

9. (Cancelled)

10. (Cancelled)

11. (Reiterated) The method of claim 1, wherein the source environment is a non-laboratory environment.

12. (Reiterated) The method of claim 1, wherein the source environment is a natural environment.

13. (Reiterated) The method of claim 1, wherein more than one microorganism is gathered from the source environment.

14. (Reiterated) The method of claim 13, wherein each organism is provided a volume of medium in a separate compartment.

15. (Amended) The method of claim 14, wherein the volume of medium is no greater than about 1 ml.

16. (Reiterated) The method of claim 14, wherein the organisms are placed in the separate compartments using flow cytometry, cell sorting, or dilution.

17. (Amended) The method of claim 1, further comprising counting at least one microorganism that grew.

18. (Amended) The method of claim 1, wherein identifying the microorganism includes hybridization of a probe to a nucleic acid molecule of the microorganism.

19. (Amended) The method of claim 1, wherein identifying the microorganism includes amplification of a nucleic acid molecule of the microorganism.

20. (Amended) The method of claim 1, wherein identifying the microorganism includes immunodetection of a molecule of the microorganism.

21. (Amended) The method of claim 1, wherein identifying the microorganism includes sequencing of a nucleic acid molecule of the microorganism.

22. (Cancelled)

23. (Amended) The method of claim 1, wherein identification of the microorganism is automated.

24. (Reiterated) The method of claim 17, wherein identifying or counting a microorganism comprises depositing cells in a two-dimensional array, such that different cultures arising from different cells each occupy a unique position in the array.

25. (Reiterated) The method of claim 17, wherein identifying or counting a microorganism comprises use of a technique that reveals a genetic or enzymatic property of the microorganisms.

26. (Reiterated) The method of claim 17, wherein a cultured strain of bacteria, called a reporter strain, is added to the medium with an unknown cell from nature, such that production of at least one compound by the unknown cell is revealed by a growth or genetic responses of the reporter strain.

27. (New) The method of claim 1, wherein the detection method comprises removal of substantially all of the medium from the cultured sample.

28. (New) The method of claim 1, wherein identifying the at least one microbial species comprises sequencing a target nucleic acid sequence of the microbial species, and comparing the sequence of the target nucleic acid to at least one known sequence of the target nucleic acid from at least one known organism.

29. (New) The method of claim 28, wherein the target nucleic acid sequence is a ribosomal RNA sequence.

30. (New) A method of isolating and identifying a microbial species from a marine source environment, comprising:

gathering from the source environment a sample suspected of containing a plurality of microorganisms that have not been cultured using standard culturing techniques;

providing a volume of culture medium based on sea water to the plurality of microorganisms in a plurality of microtiter plate compartments, such that each compartment receives no more than about three microorganisms;

incubating the plurality of microorganisms in the medium for a period of time and in an environment sufficient to result in growth of the microorganism if the medium and environment are capable of supporting such growth to produce a plurality of culture samples;

detecting growth of at least one of the plurality of microorganisms using a detect method that comprises depositing a portion of the culture sample onto a surface using a filtration manifold, , wherein growth of the microorganism comprises an increase in the number of microorganisms in the compartment to no more than about 5×10^4 cells/milliliter, and wherein

growth of the at least one microorganism indicates that the microbial species has been isolated from the source environment; and

identifying the microbial species, using a method that comprises:

sequencing a ribosomal RNA sequence of the microbial species,

comparing the sequence of the ribosomal RNA to at least one known ribosomal RNA sequence from at least one known organism; and

assigning an identity to the microbial species based on sequence similarity to the ribosomal RNA of the known organism.

REMARKS

By this amendment, an acknowledgment that this invention was made with government support under National Science Foundation Major Research Instrumentation Grant, No. OIA-9977469 is added. In addition, several obvious typographical errors in the specification are corrected. Claims 1, 15, 17-21, and 23 are amended. Claims 9, 10 and 22 are cancelled and new claims 26-30 are added.

Claim 15 is amended to correct an obvious typographical error. Claims 18-21, and 23 are amended to modify their dependency from cancelled claims. Support for the amendments and for new claims 26-30 can be found in the papers filed by the Applicant on November 21, 2000, in parent case 09/675,382.

No new matter is added by these amendments. Unless specifically stated, none of these amendments are intended to limit the scope of any claim. Upon entry of this amendment, **claims 1-8, 11-21, 23-30 will be pending in this application**. Consideration of the application and entry of the above amendments are respectfully requested.

Declaration:

In support of the following arguments, Applicants submit herewith a Declaration under 37 C.F.R. 1.132 from Inventors Stephen Giovannoni and Stephanie Connon (the Declaration). The content and significance of the Declaration are discussed throughout this response.

Claim rejections under 35 U.S.C. §102(b):

Claims 1-8, 10-17, 23, 27 and 31 were rejected in the parent case as allegedly anticipated by Jovanovich (USPN 5,756,304). In particular, it was alleged that these claims do not include any limitations that distinguish Applicants' invention from Jovanovich. Applicants traverse this rejection based on the amended claims submitted herewith and in the following arguments:

Applicants have amended claim 1 to more clearly define their invention. In particular, amended claim 1 recites that microorganisms *that have not been previously cultured using standard culturing techniques* are gathered from a source environment. In addition, amended claim 1 also recites that the growth of a microorganism is measured *as an increase in the number of microorganisms in the compartment to no more than about* 5×10^4 *cells/milliliter*. Finally, amended claim 1 recites that detection of growth is automated.

Jovanovich does not teach or suggest the presently claimed invention because a measurement of low cell density (below 5×10^4 cells/milliliter) as is currently required by the claims precludes the use of traditional methods of cell detection, such as optical density readings (Declaration at Paragraph 5). Jovanovich relies on such traditional methods to detect microorganisms (see for instance, Col. 20, lines 58-59; Col. 22, lines 38-40; Col. 23, lines 18-20). Since the lower limit of sensitivity in optical density readings is 1×10^6 bacterial cells/milliliter (see footnote 11, Exhibit C), Jovanovich teaches away from the use of optical density readings as a method of detecting microorganism densities that are lower than this threshold limit.

Moreover, as a result of Applicants' ability to detect cell densities that are orders of magnitude lower than are possible with the methods described by Jovanovich, approximately 600 cultures have been isolated to date by Applicants, including many unique cell lineages that

will be named as new species and genera by microbial systematists, which could not have been detected with the procedures described by Jovanovich (Declaration at Paragraphs 5 and 6). The detection of such low cell densities is therefore not anticipated by Jovanovich.

Applicants' claimed invention differs in another important respect from Jovanovich. Jovanovich isolates microbes from the environment using standard microbial isolation techniques and then *screens* them, whereas Applicants use high-throughput methods in order to *isolate* the microorganisms (Declaration at Paragraph 9). The term "isolate" has very different meanings in the Jovanovich patent and the current application. Thus, while it appears that Jovanovich teaches the screening of microorganisms (specifically, for bioremediation capabilities), it **does not** teach any novel techniques for the **isolation** of microorganisms from the environment. The isolation of microorganisms using high throughput methods is therefore not anticipated by Jovanovich.

Applicants' invention is distinct from Jovanovich in yet a third way, since Jovanovich describes methods that allow him to screen microorganisms for their bioremediation capabilities and optimizes the conditions under which the organisms bioremediate a particular compound (Declaration at Paragraph 9). Thus, Jovanovich screens and selects for a small subset of organisms that survive and/or adapt to the selective pressure when cultured (using standard culturing techniques) in the presence of a toxic compound. Applicants' methods, on the other hand, do not **select** for a specific type of organism. Rather, the claimed invention is directed to generating cultures that are more similar to the natural environment in both the variety and concentration of cells detected than could be achieved with standard culturing techniques (Declaration at Paragraph 9). Jovanovich is thus limited by the isolation practices that Applicants' invention was expressly designed to overcome.

Further, Applicants' have amended claim 1 to include a list of techniques by which the microbial species is identified. The Examiner has clearly stated, both in the Final Office Action (dated August 16, 2001) and in the Advisory Action (dated December 13, 2001) that "Jovanovich does not teach further identification of the microorganisms, such as amplification

and immunodetection." These limitations are now included in Claim 1, and they clearly distinguish Jovanovich.

At least these four elements distinguish the subject matter of Applicants' current claim 1 from the cited reference, and clearly distinguish that subject matter. Applicants therefore request that the rejection of claim 1 as allegedly anticipated by Jovanovich be withdrawn, in light of these arguments and the herewith submitted amendments.

Claims 2-8, 11-17, 23, and 27 all depend directly or indirectly from amended claim 1, and thereby incorporate the amended limitations regarding the isolation of microorganisms not previously cultured using standard conditions, automated detection of growth, the detection of cultures at low cell density, and specific techniques for us in the identification of the microorganisms. Therefore each of these dependent claims defines an invention that is not taught by Jovanovich. In light of all of these arguments, and the amendment to claim 1, Applicants request that the rejection of claims 1-8, 11-17, 23, and 27 under §102(b) be withdrawn.

Claim rejections under 35 U.S.C. §103(a):

According to MPEP Section 2142 (Feb. 2000) (Citing *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991).):

> To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure.

Applicants believe the Examiner has failed to meet his burden in demonstrating a *prima facie* case of obviousness against the pending claims, and provide the following arguments in support of this conclusion.

First and generally, the Examiner has provided no evidence of motivation in the art to combine any of the references that have been cited together under §103(a). The requirement for definite evidence of motivation to combine has recently been re-emphasized by the Federal Circuit in *In Re Lee*, 61 USPQ2d 1430 (Fed. Cir. 2002). Rather than providing such evidence, the Examiner appears to have cherry-picked elements from individual references, and assembled them into allegedly non-obviousness destroying sets based on hindsight, using Applicants' invention as a guide. Such hindsight is impermissible, since the "teaching or suggestion to make the claimed combination . . . must . . . be found in the prior art, and not based on [Applicants'] disclosure." (MPEP 2142) The Examiner has therefore failed to make a *prima facie* case of obviousness for any of the three rejections under §103(a), and Applicants request that these rejections be withdrawn.

Claims 18-21, 24, 25

Claims 18-21, 24, and 25 were rejected in the parent application as allegedly rendered obvious by Jovanovich in view of Sosnowski *et al.* (US 6,051,380). The Examiner alleged that Sosnowski teaches the use of an array (such as a two-dimensional array) for multi-step reactions. Applicants traverse this rejection.

Claims 18-21, 24, and 25 all depend directly or indirectly from claim 1. As discussed above, Jovanovich does not teach all of the elements of these present claims. Sosnowski *et al.* does not remedy these deficiencies.

The Examiner states that since Applicants' claims do not specify any limitations other than those of standard culturing techniques, Sosnowski *et al.* can be combined with Jovanovich to teach the method of isolating microorganisms. The limitation of culturing cells that have not been cultured using standard culturing techniques has been included in amended claim 1, and is therefore now found in all of the claims rejected over this combination of references. The

Sosnowski *et al.* reference does not teach low cell density methods for identification of cell cultures. Therefore the combination of Sosnowski *et al.* and Jovanovich does not teach or imply all of the elements of the claimed invention. Applicants therefore request that the rejection of claims 18-21, 24, and 25 under 35 U.S.C. §103 be withdrawn.

Claim 26

Claim 26, which depends indirectly on claim 1, was further rejected in the parent application as allegedly rendered obvious by Jovanovich in view of Hoover *et al.*, 1993. Applicants traverse this rejection.

The Examiner states that since Applicants' claims do not specify any limitations other than those of standard culturing techniques, Hoover *et al.* can be combined with Jovanovich to teach the method of isolating microorganisms. The limitation of culturing cells that have not been cultured using standard culturing techniques has been included in amended claim 1, and is therefore now found in all of the claims rejected over this combination of references. The Hoover *et al.* reference does not teach low cell density methods for identification of cell cultures. As a result, the combination of Jovanovich and Hoover *et al.* does not teach or imply all of the elements of the subject matter claimed in claim 26. Withdrawal of this rejection is requested.

Claims 28-30

Claims 28-30 were also rejected in the parent application as allegedly rendered obvious by Jovanovich in view of Cleveland (USPN 4,427,415) and Chee *et al.* (USPN 5,861,242). Claims 28 and 29 depend indirectly from claim 1, and claim 30 is independent. Applicants traverse this rejection also.

Jovanovich, as discussed above, does not teach all of the limitations of the claimed invention, particularly as it relates to claim 1 as presented herein. At the least, as argued above, Jovanovich does not teach or fairly imply at least four limitations of claim 1, which limitations are all incorporated into the claims depending directly or indirectly from claim 1. Neither Cleveland nor Chee *et al.*, nor a combination of these references provides explicit or implicit teachings that overcome the shortcomings of Jovanovich. In addition, as argued below, both

Cleveland and Chee *et al.* are distinguishable from the claimed invention based on the element(s) for which they are cited by the Examiner.

Cleveland teaches the use of a manifold vacuum for containment of a chemical sample (Col. 3, lines 2-3), not for the containment of microorganisms. However, Cleveland does not explicitly teach this element of the claimed invention. Nor is there any explicit or implicit teaching in Cleveland to adapt the manifold vacuum methods for use with microorganism isolation and identification. For the Examiner to conclude that it would be obvious for one of ordinary skill in the art to know to use a device as described in Cleveland for isolation and identification of microorganisms requires impermissible hindsight.

As discussed in the Declaration at Paragraph 10, the cell arraying technique used by Applicants for the identification of microorganisms is distinguishable from that described in Chee *et al.* The Chee *et al.* method immobilizes known DNA probes onto a surface, such as a two-dimensional surface. A sample of unknown DNA (such as a preparation from a single unknown cell type) is then washed over the fixed probe in order to identify the unknown DNA in the sample based on its ability to bind to the fixed probe.

This is clearly different from Applicants' claimed method, wherein unknown microorganism cells (not DNA) are immobilized onto a surface, and then a known probe is used as a wash over the cells. The cells are in this manner identified based on the ability or inability of the probes to bind to the arrayed cells, for instance by hybridization to a nucleic acid within the cells. The arraying of unknown microorganism cells onto a surface is not taught, either explicitly or implicitly, by Chee *et al.* The application of a probe washed over the cell array is also not taught. For the Examiner to conclude that ordinary skill in the art would have led one to adapt the Chee *et al.* disclosure to perform the claimed invention requires impermissible hindsight, based solely on Applicants' disclosure.

Because neither Cleveland nor Chee *et al.* implicitly or explicitly teach all elements of the method claimed in claim 1, let alone the additional elements added in dependent claims 28

and 29, these claims are clearly non-obvious over the cited references. Withdrawal of this rejection is requested.

Similarly, claim 30 now specifies that the method is isolating and identifying microorganisms that have not been cultured using standard techniques, and further that detection of growth of the microorganisms in culture is carried out when there are no more than about 5 x 10^4 cells/milliliter. Since at least these two limitations are neither taught nor suggested by Jovanovich, Chee, nor Cleveland, the combination of these three references cannot render the subject matter of claim 30 obvious. Applicants request that the rejection of this claim under §103(a) be withdrawn.

Secondary Considerations

Beyond the individual arguments presented above, Applicants invention is also clearly patentable over the cited references based on secondary considerations, in particular long felt need in the art and unexpectedly superior results.

As is discussed in the Declaration at Paragraphs 4 and 6, it has long been recognized that less than 1% of the earth's microbial life has been grown in the laboratory using standard isolation techniques. Of over 40 known prokaryotic phyla, only about half have cultured representatives and therefore, there is a continuing need to develop alternative methods of isolating microorganisms that are not amenable to standard culturing techniques. There is thus a recognized and long felt need in the art to find new methods for isolating and culturing these intractable strains in the laboratory.

Applicants' methods easily detect growth in a culture chamber even where the introduced cell has divided only a few times in the chamber and may then only number 200 cells (Declaration at Paragraph 5). Since these methods can detect cell densities that are orders of magnitude lower than are possible with the standard methods employed by Jovanovich, 600 new cultures have been isolated. This has satisfied the long felt need to isolate intractable cultures (Declaration at Paragraph 6). In addition, the isolation and culturing of this many new,

previously uncultured microorganisms is dramatic and unexpectedly superior compared to all known prior art methods of isolating microorganisms.

CONCLUSIONS

Applicants believe that the claims submitted herewith are in condition for allowance. If for any reason the Examiner believes that a telephone conference would expedite allowance of the claims, please telephone the undersigned at (503) 226-7391.

Respectfully submitted,

KLARQUIST SPARKMAN, LLP

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TMH/AC:dsh:tjj 05/10/02 100615
PATENT

Attc · Reference Number 245-62107
Application Number 10/053,243

Marked-up Version of Amended Specification and Claims
Pursuant to 37 C.F.R. §§ 1.121(b)-(c)

In the Specification:

Please amend the specification to include the following statement regarding governmental funding:

ACKNOWLEDGMENT OF GOVERNMENT SUPPORT

This invention was made with government support under National Science Foundation Major Research Instrumentation Grant, No. OIA-9977469. The government has certain rights in this invention.

Please replace the paragraph at Page 9, lines 16-23, with the following:

In certain embodiments, cells can also be cultured on conventional growth media, to compare growth rates, ~~cutlurability~~culturability, or like parameters. For instance, a sample of the raw source material may be used as a starting culture for growth on nutrient agar plates. In other instances, a portion of some or all of the microbial cultures that show growth in the micro-culture compartments (*e.g.*, the compartments of a microtiter dish) can be used to inoculate traditional medium (either liquid medium or nutrient agar plates, for instance), in order to examine whether the microbes will ~~growth~~ grow under traditional conditions.

Please replace the paragraph at Page 10, lines 12-19, with the following:

Within an array, each arrayed cell sample or mixture of cells is addressable, in that its location can be reliably and consistently determined within ~~the~~ at least the two dimensions of the array surface. Thus, in ordered arrays the location of each cell sample is assigned to the sample at the time when it is spotted onto the array surface and usually a key is provided in order to correlate each location with the appropriate "target" cell sample. Often, ordered arrays are arranged in a symmetrical grid pattern, but samples could be arranged in other patterns (*e.g.*, in radially distributed lines or ordered clusters).

Please replace the paragraph at Page 11, line 29 through Page 12, line 3, with the following:

Sample spots on macroarrays are of a relatively large size, for instance large enough to permit detection of a ~~hybridiziation~~ hybridization signal without the assistance of a microscope or other sophisticated enlargement equipment. Thus, spots may be as small as about 0.1 mm across, with a separation of about the same distance, and can be larger. Larger sample spots on macroarrays, for example, may be about 0.5, 1, 2, 3, 5, 7, or 10 mm across. Even larger spots may be larger than 10 mm (1 cm) across, in certain specific embodiments. The array size will in general be correlated to the size of the sample spots applied to the array, in that larger spots will usually be found on larger arrays, while smaller spots may be found on smaller arrays. This correlation is not necessary to the invention, though.

Please replace the paragraph at Page 21, lines 6-11, with the following:

Certain examples of automated array readers (scanners) will be controlled by a computer and software programmed to direct the individual components of the reader (*e.g.*, mechanical components such as motors, analysis components such as signal interpretation and background subtraction). Optionally software may also be provided ~~reader~~ to control a graphic user interface and one or more systems for sorting, categorizing, storing, analyzing, or otherwise processing the data output of the reader.

Please replace the paragraph at Page 24, lines 25-28, with the following:

To prepare the frozen dilution cultures for lysis and concentration, the tubes were placed into a 95 °C heat block for five minutes, then put in –80 °C for 15 minutes. This process, which helps ~~unsure~~ insure cell lysis, was repeated once, and the tubes finally thawed at room temperature.

In the Claims:

1. (Amended) A method of isolating and identifying a microbial species from a source environment, comprising:

gathering from the source environment a sample suspected of containing at least one microorganism ~~from the source environment~~that has not been cultured using standard culturing techniques;

providing a volume of culture medium to the microorganism in at least one microtiter plate compartment;

incubating the microorganism in the medium for a period of time and in an environment sufficient to result in growth of the microorganism if the medium and environment are capable of supporting such growth to produce a culture sample, wherein growth of the microorganism comprises an increase in the number of microorganisms in the compartment to no more than about 5×10^4 cells/ milliliter; ~~and~~

detecting growth of the microorganism using an automated detection method that comprises removing a portion of the culture sample and depositing the portion onto a surface, wherein growth of the microorganism indicates that the microbial species has been isolated from the source environment; and

identifying the microbial species, wherein identifying the microorganism includes hybridization of a probe to a nucleic acid molecule of the microorganism amplification of a nucleic acid molecule of the microorganism; immunodetection of a molecule of the microorganism; sequencing of a nucleic acid molecule of the microorganism; or a combination of two or more thereof.

15. (Amended) The method of claim 14, wherein the volume of medium is no greater than about 1 m~~L~~l.

17. (Amended) The method of claim 1, further comprising ~~identifying or~~ counting at least one microorganism that grew.

18. (Amended) The method of claim ~~17~~1, wherein identifying the microorganism includes hybridization of a probe to a nucleic acid molecule of the microorganism.

19. (Amended) The method of claim ~~17~~1, wherein identifying the microorganism includes amplification of a nucleic acid molecule of the microorganism.

20. (Amended) The method of claim ~~17~~1, wherein identifying the microorganism includes immunodetection of a molecule of the microorganism.

21. (Amended) The method of claim ~~17~~1, wherein identifying the microorganism includes sequencing of a nucleic acid molecule of the microorganism.

23. (Amended) The method of claim ~~17~~1, wherein identification of the microorganism is automated.